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TITLE: Modeling NF2 Tumors for Drug Screening Using Induced Pluripotent Stem Cells

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14. ABSTRACT
Neurofibromatosis type 2 (NF2) is an autosomal dominant genetic disorder with a penetrance of nearly 100% by age 601. NF2 is characterized by multiple tumors to the nervous system and skin, with the most common being a vestibular schwannoma2. Hearing loss is the most common presenting symptom in adults, becoming life-threatening as the tumor impinges on vital structures1. Patients demonstrate considerable heterogeneity in tumor onset and growth and novel tools to explore patient variability are needed. The complex local microenvironment consists of the nerve, schwannoma and vasculature, largely endothelial cells, is not typically modeled as a composite in vitro. Animal models capture this crosstalk but are limited (e.g. Avastin is specific to human, but not murine VEGF)3. The development of a 3D in vitro NF2 model using iPSCs to generate both Schwann cells and endothelial cells would enable screening of novel therapeutics, as well as a better understanding of current and novel treatments.

15. SUBJECT TERMS-

None provided

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Abstract for Form 298

NF2 is a genetic disorder which results in the increased propensity to form Schwannomas. Current treatment options are limited; chemotherapy is not appropriate due to the slow tumor growth and tumors are often considered inoperable due to their number, location and risk of nerve damage. Thus, there is a need for novel therapies with low toxicity, as patients tend to be younger requiring long-term treatment. NF2 schwannomas most commonly are found on the vestibulocochlear nerve and their frequency decreases in lower parts of the body, including the lower limbs. The vestibulocochlear nerve fires at a much higher frequency than more distal nerves. This raises the hypothesis that higher electrical activity contributes to the formation of Schwannomas. In this project we are investigating the effect of electrical activity on tumor formation in in vitro models of Schwannoma. We will use human Schwannoma cells and induced pluripotent stem cells to model the disease in vitro and investigate the impact of electrical stimulation on secretion of cytokines that contribute to angiogenesis, as well as the effect of antiangiogenic drug treatments. In this project period we have identified electrical activity conditions that do not adversely affect Schwannoma cell viability. Notably, we have identified frequencies that significantly increase tumor cell growth, and that are related to the expected neural frequencies of the vestibulocochlear nerve. This finding is significant as it indicates that the higher frequency electrical stimulation could be in part responsible for the increased growth of tumors in this location. In preparation for iPSC modeling, we have established a protocol to produce Schwann cells from iPSCs. In addition, we have arranged with Dr. Scott Plotkin to obtain samples from NF2 patients in order to generate iPSCs; the IRB approvals are still on-going.

1. Introduction

Neurofibromatosis type 2 (NF2) is a genetic disorder with mutations in the tumor suppressor protein Merlin (Fong et al. 2011). Current treatment options are limited; chemotherapy is not appropriate due to the slow tumor growth and tumors are often considered inoperable due to their number, location and risk of nerve damage (Lim et al. 2014; Balasubramaniam et al. 2007; Evans 2009). Thus, there is a need for novel therapies with low toxicity, as patients tend to be younger, requiring long-term treatment. Avastin, a human vascular endothelial growth factor (VEGF) antibody, has exhibited some efficacy in the clinic and is generally well tolerated (Fong et al. 2011). Avastin counteracts VEGF, which acts on endothelial cells to form new blood vessels and promotes endothelial proliferation, migration, and survival. VEGF has been shown to be expressed by Schwann cells (Brushart et al. 2013). The loss of a functional merlin protein in NF2 upregulates VEGF, which spurs angiogenesis (London & Gurgel 2014). NF2 schwannomas most commonly are found on the vestibulocochlear nerve that fires at a much higher frequency (0.5-10 kHz) than the more distal nerves (in the tens of Hz) (Carney & Yin 1988; Searchfield et al. 2004). Schwann cells are known to be responsive to electrical activity, and differences in activity may alter cytokine production in the NF2 microenvironment, contributing to angiogenesis and tumor growth (Koppes et al. 2011; Koppes et al. 2013; Koppes et al. 2014). In this project, we aim to investigate the effect of electrical activity on an NF2 Schwannoma cell line, quantifying cell proliferation and cytokine production, and will use human induced pluripotent stem cells (iPSCs) to generate patient-specific NF2 Schwann and endothelial cells to model the disease in vitro and investigate drug treatments.

2. Keywords

Neurofibromatosis type 2, induced pluripotent stem cells, angiogenesis, electrical stimulation, personalized medicine

3. Accomplishments

Major goals of the project

Aim 1: Generate 4 patient-specific iPSC Avastin-responsive and control cell lines to produce human Schwann cells and endothelial cells for use in the 3D microenvironment.

Aim 2: Develop a 3-dimensional (3D) model of the acoustic nerve microenvironment to evaluate changes in Schwann cell proliferation and angiogenesis in response to tonic electrical stimulation in the vestibulocochlear nerves.

Aim 3: Evaluate Avastin in the 3D model of the acoustic nerve microenvironment using NF-2 patient derived Schwann cells and endothelial cells; test combined with anti-IGF-1R and/or anti-PDGFR.

Table 1 Summary of completion relative to approved SOW

TASKS	Timeline (months)	Site 1	Site 2	% completion NA= Not applicable in this project period
Specific Aim 1	1-18 mo.			
Local IRB/ISCRO and HRPO Approval	1-6	Temple	Thompson	95%
Derive PSC lines from NF2 (4 NF2 patients); controls already in hand	6-18	Temple		0% (depends on IRB approval; see explanation below)
NF2 Genetic Analysis	6-14	Temple		0% (depends on IRB approval
Differentiate iPSC into Schwann cells, purify, characterization	9-16	Temple		80%
Modeling Schwannoma loss of NF2 via Knockdown experiments	15-18	Temple		*NA*
Differentiate iPSC into endothelial cells, purify, characterization	10-18	Temple		20% (Training has occurred)
AIM 1: Milestone(s): >Develop and characterize NF2 iPSC lines >Differentiate to Schwann cells or Endothelial cells	18 mo	Temple		NA
Specific Aim 2	1-18 mo			
HEI-193 response to electrical stimulation: > magnitude > frequency	1-8 mo		Thompson	100%
Development of an NF2 in vitro model to examine both changes in Schwannoma proliferation/viability and changes in angiogenesis	1-8 mo		Thompson	50%
Development changes in Schwannoma proliferation/viability and changes in angiogenesis due to tonic electrical stimulation	9-18 mo	Temple	Thompson	50%
AIM 2Milestone(s): >Develop a 3D model of vasculature and Schwannoma to quantify changes in Schwannoma proliferation and angiogenesis >Evaluate HEI-193 Schwannoma response to electrical stimulation and any electrically mediated changes to angiogenesis using HUVEC as model human lines	18 mo	Temple	Thompson	NA
Specific Aim 3	19-34			NA
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to Co-culture	19-22	Temple	Thompson	
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to electrical stimulation	20-23	Temple	Thompson	
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to electrical stimulation and Avastin	23-26	Temple	Thompson	
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to electrical stimulation and Avastin + anti-IGF	26-29	Temple	Thompson	
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to electrical stimulation and Avastin + anti-PDGFR	28-31	Temple	Thompson	
Sensitivity of iPSC derived Schwann cell proliferation and Endothelial angiogenesis to electrical stimulation and effective combinations of drugs (Avastin, anti-IGF, anti-PDGFR)	32-35	Temple	Thompson	

AIM 3Milestone(s) Achieved: Evaluate sensitivity of iPSC Schwann cell proliferation and iPSC endothelial angiogenesis to one or more of the following in the novel 3D platform: > tonic electrical stimulation >Avastin >Anti-IGF >Anti-PDGFR	9* Please see note below	Temple	Thompson	
Manuscript preparation	18-36	Temple	Thompson	

*****The timeline for these milestones above in the SOW are given at 9 months, however this is an error on our part as the timeline for aim 3 does not start until month 19. Based on the aim 3 work proposed, the timeline for achieving these milestones should be 35 months.

Accomplishments towards these goals for this project period.

Aim 1.

1.1 **Agreements and IRB approval**

We have arranged with Dr. Scott Plotkin to obtain NF2 samples from patients, and have been working with him and the IRB at Massachusetts General Hospital (MGH) and the IRB at Albany Medical College (AMC) to complete the paperwork required. We have now obtained IRB approval from MGH. The IRB application had then to be re-written according to Albany Medical College IRB requirements. The IRB application has been submitted to Albany Medical College IRB. Albany Medical College IRB has asked MGH IRB for a reliance agreement, which means they would be the sole IRB on file responsible for the project. We are still waiting to hear if MGH will grant this reliance agreement. A summary of the points of contact and paperwork submissions are given below, but do not include the numerous emails and phonecalls made to spur the process. We believe that we are now at the final step, awaiting MGH IRB's reliance agreement.

Summary of contacts to obtain IRB approval.

April 21, 2015: first draft of the protocol was sent to Dr Plotkin at Massachusetts General Hospital (MGH) for editing.

May 3, 2016: HRPO request for documents was received

May 9, 2015: Forwarded HRPO request for documents to MGH

May-July 2015: Numerous discussions with Dr Plotkin and MGH staff on protocol, responsible parties, required documents etc

June 23, 2015: Protocol and scientific review submitted to Albany Medical Center (AMC) IRB minus the clinical documents required from MGH

July-August 2015: Continued discussions and status update requests re: Massachusetts General Hospital/Brigham and Women's Hospital (MGH/BWH) IRB submission.

August 31, 2015: Clinical research assistant brought on board to facilitate protocol submission and initial discussions to finalize protocols and required documents

September-November 2015: MGH submission package was written and edited. Final protocol agreed upon November 23.

December 16, 2015: Submission accepted with MGH/BHW's IRB

February 22, 2016: First round of revisions from MGH/BWH's IRB

April 25, 2016: 2nd round of revisions/requests for comment from MGH/BWH's IRB

May 12, 2016: Approval issued from MGH

June-July 2016: Initial protocol submitted needed to be rewritten subject to MGH/BWH's approvals

August 3, 2016: Protocol resubmitted to AMC's IRB

September 6, 2016: AMC contacted MGH/BWH's IRB for a reliance agreement to have MGH/BWH's IRB serve as IRB for the entire study. The outcome of this request is still pending.

1.2 Differentiation of pluripotent stem cells into Schwann cells

We have successfully trained in producing both endothelial cells and Schwann cells to prepare for the differentiations required. Further, we have successfully completed a pilot study of the Schwann cell differentiation protocol using wild-type adult iPCSs. We are using the differentiation protocol kindly provided to us by Dr. Studer (see Training and Professional Development). Fig. 1 shows a representative fluorescence image of the resulting differentiated cells at day 36 of the differentiation protocol. Notice that the majority of the cells are stained for S100β, a Schwann cell marker.

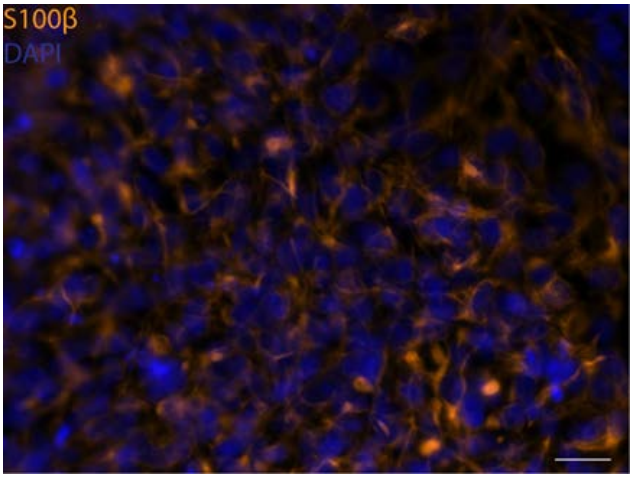

Fig. 1. Wild-type adult iPSCs at day 36 of differentiation. Cells were stained for S100β (orange). Nuclei are stained with DAPI (blue). Scale bar: 50 μm.

Aim 2.

Development of the 3D model:

2.1 Effect of tonic stimulation on Schwannoma cells

In order to develop a 3D model of the acoustic nerve microenvironment we sought to determine the effect of tonic electrical stimulation on Schwann cell proliferation. We used the HEI-193 immortalized cell line which is the only NF2 human Schwannoma cell line, obtained under appropriate MTA.

Cells were seeded on glass coverslips at a density of $5x10^4$ cells/cm^2 or $2.5x10^4$ cells/cm^2 (for stimulation periods of less or more than 24 hours, respectively) and cultured overnight. The coverslips were then placed in a custom rectangular poly(dimethylsiloxane) (PDMS) chamber with a defined geometry. Biological-grade electrodes were placed at the two ends of the culture chamber to measure the voltage across the chamber using LabView. AC stimulation was applied to Schwannomas via a pulse generator.

HEI Viability: NF2 Schwannoma cell viability was quantified immediately after stimulation using ethidium homodimer III (EthD-III), which stains dead cells. Cells were subsequently fixed using paraformaldehyde and stained with 4',6-diamidino-2-phenylindole (DAPI), which stains all cells. Cell viability was calculated by imaging the entire coverslips with a Zeiss Axiobserver Z.1 fluorescent microscope, and measuring the ratio of EthD-III+DAPI+/DAPI+ pixels automatically using ZEN 2 software. The cells were stimulated at high frequency (1 kHz) and low frequency (20 Hz) for 0.1 ms with a range of stimulation magnitudes from 0-100 mV/mm. Fig. 2 shows that HEI-193 viability is not significantly affected by any of the different stimulation experimental conditions we used.

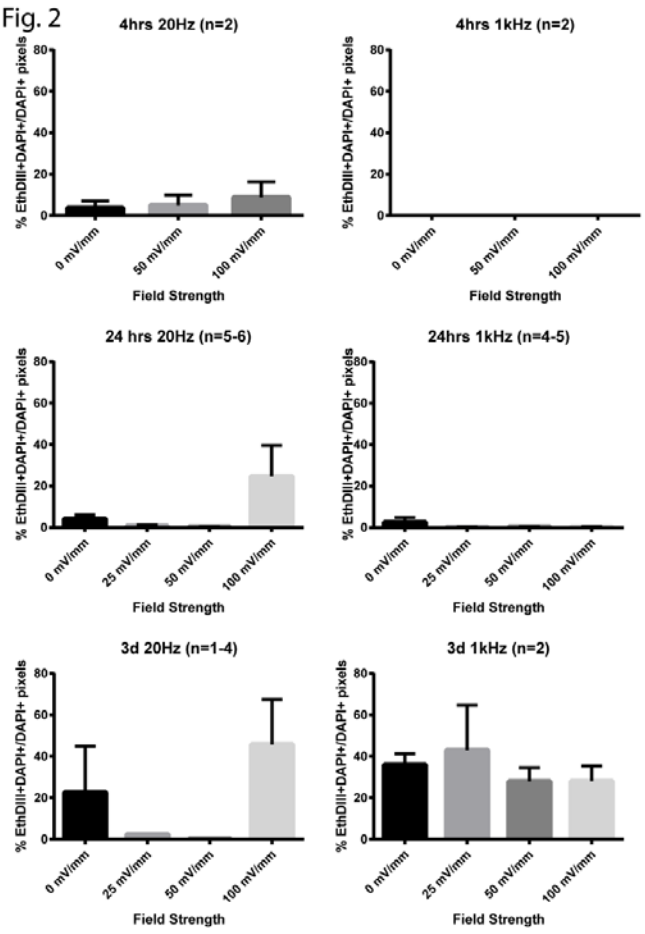

Fig. 2. Electrical stimulation does not affect HEI-193 cell viability. Cells were stimulated either for 4 hours (top panels), 24 hours (middle panels), or three days (bottom panels), with a frequency of either 20 Hz (left panels) or 1 kHz (right panels). Within these conditions, the effect of the strength of the electrical field applied (mV/mm) was also tested. None of the stimulation conditions displayed significantly affected the viability of the cells when compared to unstimulated controls (0 mV/mm) ($p>0.05$, 1-way ANOVA, the number of replicates are given in parentheses at the title of each graph).

HEI Proliferation: NF2 Schwannoma cell proliferation was estimated by measuring the area of the coverslips covered by DAPI and comparing it to unstimulated controls (using ZEN 2 software) (Fig. 3). None of the stimulation conditions tested significantly

affected HEI-193 proliferation. However, at 50 mV/mm field strength and 1 kHz frequency for 4 hours, the proliferation of HEI-193 cells showed a trend towards being higher than that of controls, while longer stimulations under the same conditions produced more variable results.

It is likely that stimulating for 4 hours and fixing immediately to obtain results is not sufficient time to detect possible effects on proliferation. In order to investigate the effect of longer incubation times after stimulation, additional experiments were performed. HEI-193 cells were plated at low density and an experimental field strength of 50 mV/mm applied, but after the end of the stimulation period the medium was replaced with fresh growth medium and the cells were cultured for an additional 3 days. Cells were subsequently fixed as described above and stained with DAPI, and HEI-193 proliferation was then calculated. Fig. 4 shows that 3 days after application of 50 mV/mm field strength for 4 hours, HEI-193 cells proliferated significantly more compared to unstimulated controls. Furthermore, we have shown that 1 kHz electrical activity (similar to that of the vestibulocochlear nerve) causes significantly more proliferation of Schwannoma cells than 20 Hz (similar to that of more distal nerves) (Fig. 4). Hence, under these conditions, electrical stimulation appears to have a growth promoting effect on Schwannoma cells. Why this was not observed when the cells were treated with 3 days of continuous stimulation is not clear. However, the in vivo environment is much more complex, and it is possible that additional factors, or variations in nerve activity, interact with the Schwann cells to affect proliferation. Nevertheless, the fact that we have discovered an electrical stimulation treatment that does affect Schwannoma cell proliferation, and is in the order of vestibulocochlear nerve activity, allows us to move ahead with the co-culture model.

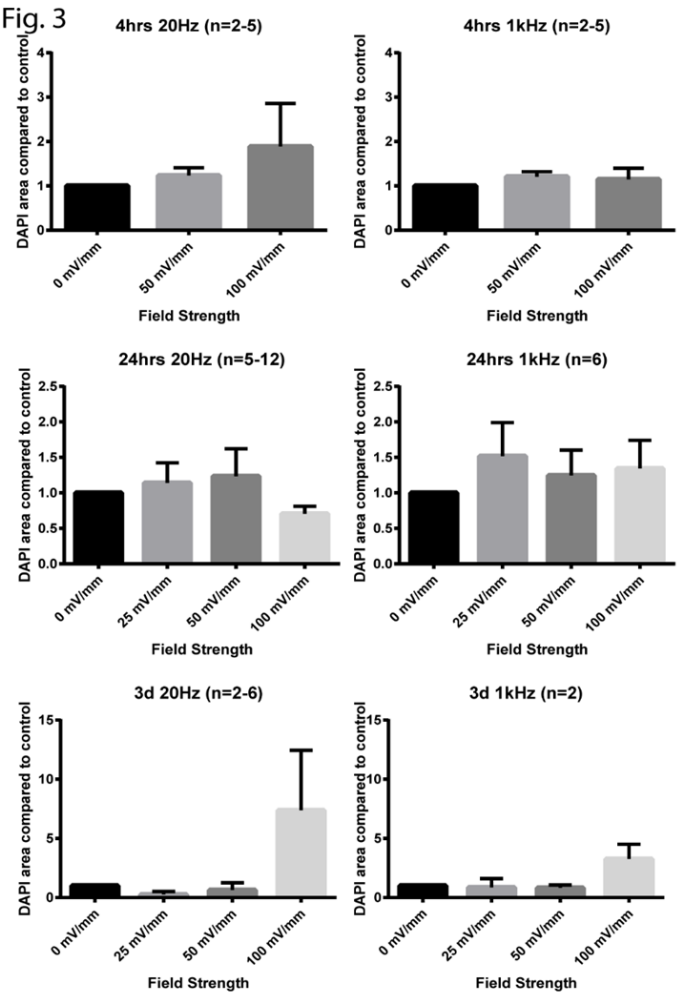

Fig. 3. Effect of electrical stimulation on HEI-193 proliferation. Cells were stimulated either for 4 hours (top panels), 24 hours (middle panels), or three days (3d) (bottom panels), with a frequency of either 20 Hz (left panels) or 1 kHz (right panels). Within these conditions, the effect of the strength of the electrical field applied (mV/mm) was also tested. None of the stimulation conditions displayed significantly affected the proliferation of the cells when compared to unstimulated controls (0 mV/mm) ($p>0.05$, 1-way ANOVA, the number of replicates are given in parentheses at the title of each graph).

2.2 Media Selection for co-culture model

After determining appropriate stimulation conditions that enhance the proliferation of HEI-193 cells, our goal is to co-culture these cells with endothelial cells in a 3D model of the acoustic nerve microenvironment. In order to complete this task, we had to determine appropriate culture conditions that would not adversely affect the growth of either HEI-193 or endothelial cells. We used human umbilical vein endothelial cells (HUVECs) as model endothelial cells. We cultured the same number of cells for each cell line individually for 4 and 8 days in vitro (DIV) using either the manufacturer-specified growth medium or a 1:1 combination culture medium. After the specified time, cells were fixed and stained for DAPI as mentioned above. Cell proliferation was calculated by measuring the DAPI+ area of the coverslips using ZEN 2. Fig. 5 shows that while the two cell lines grew as well in both media for the first 4 DIV, HEI-193 cell line growth was significantly stunted by 8 DIV in the combination medium. Hence more investigation to establish the optimal culture medium to support both cell types is required.

Training and Professional Development Opportunities

During this project period, Dr. Maria Apostolopoulou received training in electrical stimulation of cells with Dr. Thompson. She also received training in the maintenance and differentiation of iPSCs with Dr. Temple. The differentiation of iPSCs into Schwann cells and endothelial cells is specialized, so Dr. Apostolopoulou also received training with two expert labs: the differentiation of stem cells into Schwann cells during a visit to Dr. Studer's lab in the Memorial Sloan Kettering Cancer Center, and the differentiation of stem cells into endothelial cells during a visit to Dr. Daylon James' lab at Weill Cornell Medical College. Dr. Apostolopoulou also attended the International Society for Stem Cell Research conference in San Francisco in June 2016, where she had the opportunity to learn about research progress in iPSC modeling of diseases of the nervous system.

Dissemination of Results to Communities of Interest

Dr. Temple attended the Faster Cures meeting on Neurofibromatosis and also the Children's Tumor Foundation strategic planning meeting, and at both was able to discuss the state of the field and the status of iPSC modeling of Neurofibromatosis. According to other trainees, patient-derived NF2 iPSCs are highly desirable in order to study the disease.

During this funding period, the Regenerative Research Foundation, one of the grantee institutions, held an open house which the local community was encouraged to attend. During this event, scientists interacted with the community to inform them of ongoing projects, and enhance public understanding and interest in science.

Next Reporting Period Plans

As outlined above, we are expecting appropriate NF2 donor tissue to develop patient-specific iPSC lines which we will then differentiate into patient specific Schwann cells and endothelial cells. We have already established the effect of a period of electrical stimulation on the HEI-193 Schwannoma cell line at physiological-relevant levels and we plan to assay cell conditioned medium after stimulation with a human cytokine array. This will identify soluble factors that might change as a result of the electrical stimulation, which could impact Schwannoma cell proliferation and also angiogenesis. Further, this

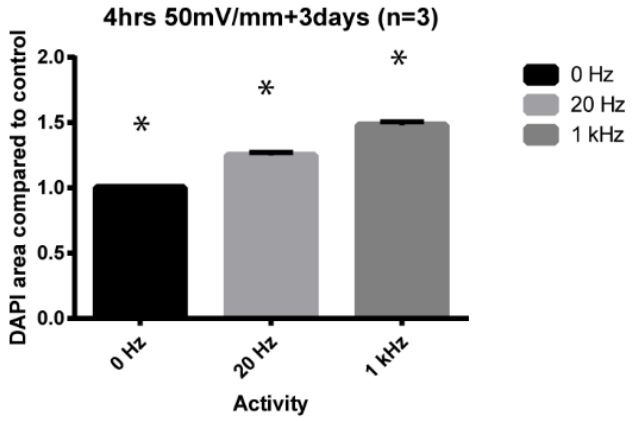

Fig. 4. Electrical activity of 1 kHz causes significantly more proliferation than 20 Hz. HEI-193 cells were stimulated for 4 hrs in a 50 mV/mm field, with either 20 Hz or 1 kHz activity (or left unstimulated as controls (0 Hz)). Electrical activity significantly affected cell proliferation ($p<0.05$; 1-way ANOVA, the number of replicates are given in parentheses at the title of the graph). Furthermore, 1 kHz caused significantly higher proliferation than 20 Hz, which in turn caused higher proliferation than no activity (control, 0 Hz) ($p<0.05$; Tukey's multiple comparisons test).

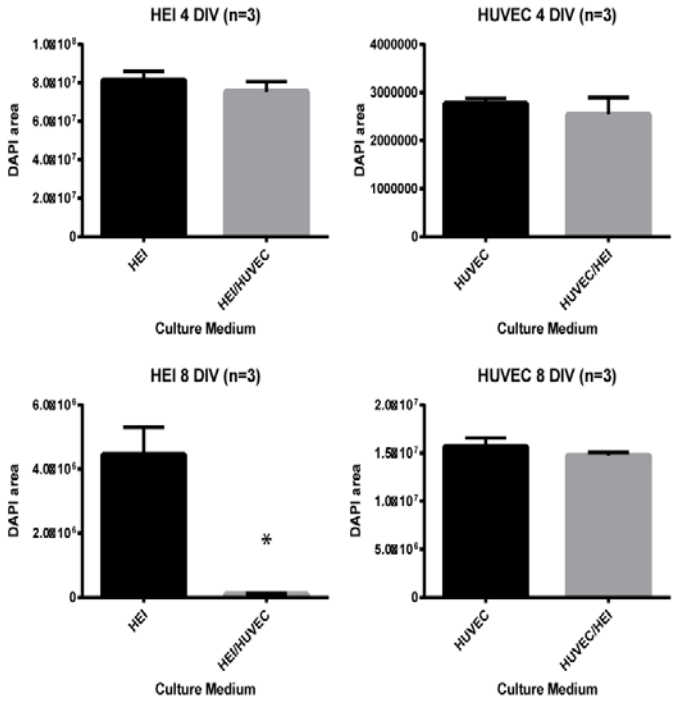

Fig. 5. HEI-193 cells show significantly reduced proliferation in combination media. Equal cell numbers of each cell line (HEI-193, left panels; HUVEC, right panels) were plated on coverslips and grown for 4 DIV (top panels) or 8 DIV (bottom panels) using two different growth media (HEI and HUVEC are the manufacturer-suggested growth media, while HEI/HUVEC is a 1:1 combination medium of the two). HUVEC cells grew equally well in both media up to 8 DIV. On the other hand, HEI-193 grew equally well on both media up to 4 DIV, after which their numbers were significantly reduced ($p<0.05$; unpaired 2-way t-test with Welch's correction).

analysis of the cytokines produced could identify key agents influencing cell proliferation so that we can gain insight into how to counteract the production of such factors to minimize Schannoma growth. We will also finish the design of the 3D chambers which will serve as models of the acoustic microenvironment. Once we have patient-derived cells and the chambers, we will test the effect of Avastin, anti-IGF-1R, anti-PDGFR and other factors of interest identified by the human cytokine array mentioned above.

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4. Impact

Impact on the Development of the Principal Disciplines of the Project

Our finding that electrical stimulation levels comparable to that of the vestibulocochlear nerve increases Schwannoma cell proliferation more than distal-nerve-level stimulation is novel and likely to expand the research field in Schwannomas to include analysis of the effects of stimulation in basic research or therapeutic approaches.

Impact on Other Disciplines

Electrical stimulation might affect the growth of other tumors in close proximity to neurons. Our results provide incentive to examine the effect of electric stimulation on such tumors, such as occur due to NF1 mutations. Studies to determine if electrical stimulation has an impact on normal cells or cells following injury may be relevant for expanding cells ex vivo for cell-mediated therapy or promoting regeneration in vivo.

Impact on Technology Transfer

Nothing to report.

Impact on Society beyond Science and Technology

Nothing to report

5. Changes/Problems

The applications for the IRB approvals were more complex and time-consuming than originally anticipated and this has led to delays. However, we believe we are close to completing this paperwork, and we hope to have the go-ahead to start collecting NF2 patient tissue samples (Dr. Plotkin) in order to make iPSC lines shortly.

In our original submission, we had proposed to use the Live/Dead assay to determine cell death, and the Cy-Quant DNA proliferation assay. We have replaced these assays with the EthD-III/DAPI and DAPI staining, respectively. The reason for this change, in the case of the Live/Dead assay, was because EthD-III/DAPI preserved the natural state of the stained cells more accurately, as determined by phase microscopy. The Cy-Quant DNA proliferation assay was replaced with DAPI staining as we were able to perform this in a more timely manner.

We originally suggested that we would make viability and cell number measurements by imaging 8 regions along the chambers of cells. Since submission of our proposal, the Regenerative Research Foundation has procured a Zeiss Axiobserver Z.1 fluorescent microscope, which simplified whole- coverslip imaging. Whole coverslip imaging avoids image sampling errors, so this is a better approach.

While testing an appropriate culture medium for co-culturing Schwannoma and endothelial cells, we found that HEI-193 cells did not grow as well in a 1:1 combination media for 8 DIV. We will work on establishing an alternate growth medium for the acoustic nerve modeling chambers, or otherwise limit our experiments up to 4 DIV.

6. Products

Nothing to report.

7. Participants and Other Collaborating Organizations

Individuals Worked on the Project

Name: Sally Temple, PhD
Position: Principal Investigator
Months Worked: 1 calendar month
Contribution: Overall direction of the research activities.
Support: CDMRP NF140040 award.

Name: Deanna Thompson, PhD
Position: Co-Investigator
Effort: 1 summer month
Contribution: Development and testing of the 3D NF model.
Support: CDMRP NF140040 award.

Name: Maria Apostolopoulou, PhD
Position: Postdoctoral Fellow
Months Worked: 12 calendar months
Contribution: Design and conduct of the planned experiments.
Support: Van Auken Postdoctoral Research Fellowship

Changes in Support of PD/PIs or other Senior/Key Personnel

Maria Apostolopoulou received a Van Auken Postdoctoral Research Fellowship.

Dr. Temple received an award from the NYS-DOH for spinal cord research. This new funding is highlighted in the revised funding table included in the Appendix section.

Other Organizations Involved as Partners

Name: Rensselaer Polytechnic Institute
Address: 110 Eighth Street
Troy, NY 12180-3522
Facilities: Dr. Thompson laboratory is located at RPI.
Collaboration: Dr. Thompson serves as the Co-Investigator on this collaborative project. Experiments are conducted at both the principal site, Regenerative Research Foundation, and the facilities at RPI.

8.Special Reporting Requirements

None

9. Appendix

Sally Temple, PhD –Other Support

ACTIVE

R01NS074047 (Temple) 09/15/2011 – 08/31/2017 1.2 calendar
NIH/NINDS $ 1,952,458
Asymmetric cell division of CNS progenitor cells
The aim of this study is to identify RNA molecules segregated during asymmetric cell divisions in the developing cerebral cortex. Dr. Scott Tenenbaum is co-investigator through an agreement with the College of NanoScience and Engineering - University at Albany.

R01NS076709 (Cohen) 09/01/2011-08/31/2017 0.6 calendar
NIH/NINDS
Analyzing Neural Stem Cell Clonal Development
Dr. Temple serves as a co-investigator through a subcontract arrangement with Drexel University. The goal is to develop automated image analysis techniques of embryonic cortical stem cell lineages.

R01EY022079 (Temple) 02/01/2012 – 01/31/2017 1.2 calendar
NIH/NEI $ 2,143,728
Defining the molecular mechanisms underlying human RPE plasticity
This project will investigate the mechanism by which retinal pigment epithelium (RPE) undergoes metaplasia and acquires abnormal cell fates and the relevance these changes to retinal disease. Dr. Joanna Wysocka participates as a Co-Investigator through a subaward with Stanford University.

R01AG041861 06/01/2012-05/31/2017 1.2 calendar
NIH/NIA $ 2,072,960
Quantifying Changes in Neural Stem Cell Lineages in the Aging Niche
This study will quantify the effect of aging on adult neural stem cells due to aging of the vascular niche. Dr. Andrew Cohen serves as a Co-Principal Investigator through a subaward agreement with Drexel University.

W81XWH-15-1-0152 09/15/2015 – 09/14/2018 1.2 calendar
CDMRP-USAMRMC $ 674,211
Modeling NF2 Tumors for Drug Screening Using Induced Pluripotent Stem Cells
We will develop novel NF2 iPSC lines for use in investigations of Schwann cells – endothelial cells relevant to NF2 disease and testing of patient-specific drug therapies.
All potential conflicts of time commitment, and budgetary or scientific overlap will be resolved through negotiation with NINDS or other awarding agency prior to accepting the award.

C028504 (Temple) 03/01/2013 – 02/28/2017 3.6 calendar
NYS-DOH NYSTEM $ 10,805,636
Retinal Stem Cell Consortium
The goal of this project is to bring together a diverse group of researchers to accelerate translational and preclinical research toward clinical applications of stem cell research for treatment of eye disease.

Not applicable (Temple) 10/01/2013-09/30/2017 0.6 calendar
Macula Vision Research Foundation $ 300,000
Reprogramming the human retinal pigment epithelial stem cells (RPESCs) into photoreceptor cells
We propose to explore the plasticity of RPE stem cells to make neural retinal progeny including photoreceptor cells. Such induced retinal and photoreceptor progenitor cells will serve as novel platforms for studying photoreceptor degenerative diseases and potentially as a novel source tissue for transplantation.

DOH01-C30605GG-3450000 (Temple) 11/01/2015 – 10/31/2018 1.8 calendar
NYS-DOH SCIRB $ 1,097,684
Sustained delivery of IL10 and SHH to promote spinal cord regeneration after injury
Here we propose to test whether a combination of sustained IL-10 and sustained SHH delivered via microbeads to the injury site will counteract inflammatory processes, promote a regenerative environment and improve recovery better than either alone. This is a collaborative project involving Aileen Anderson, Ph.D. through a subaward with UC Irvine.

PENDING
R35NS097277 (Temple) 11/01/2016 – 10/31/2024 6.0 calendar
NIH/NINDS $ 4,760,000
Defining Characteristics of Cortical Progenitor Cells over Time in Mouse and Human
This NINDS R35 proposal will support the ongoing research in our lab to explore the interaction of environmental factors on cortical progenitor cells, aided by the ability to rapidly quantify changes in proliferation, division mode and differentiation using time-lapse analysis. Recent work has identified a panel of candidate niche molecules secreted by the choroid plexus that could interact with receptors expressed on neural progenitors, which we propose to examine in vitro and in vivo, in mouse and human.

NOTE:
All potential time commitment, budgetary, and/or scientific overlap conflicts will be resolved through negotiation with NINDS or other awarding agencies prior to accepting this pending award.